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REGULATION OF CORPORA ALLATA IN FEMALES OF PYRRHOCORIS APTERUS (HETEROPTERA) (A MINI-REVIEW)

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SUMMARY

Mechanisms for the transduction of photoperiodic and food signals to the corpus allatum (CA) are described. The focus of this paper is on the control of the CA by the brain in adult females of the firebug, Pyrrhocoris apterus. By using surgical interventions to the neuroendocrine complex of brain–subesophageal ganglion–corpora cardiaca–CA (BR–SG–CC–CA) in vivo and in vitro we were able to identify two regulatory pathways. (1) Slow regulation of the CA activity (stimulation or inhibition) needs a relatively long period of time to be accomplished (several d) in vivo and is associated with changes of the gland cell volume and ultrastructure. The stimulated or inhibited activity of the CA is maintained during short-term incubation of the isolated CA in vitro. (2) Fast inhibition of the CA activity is reversible during short-term incubation in vitro; the CA can be switched from lower to higher activity and vice versa, depending on the presence or absence of the BR–SG in the medium. Both slow and fast regulatory factors originate in the pars intercerebralis of the brain and in intact neuroendocrine complex they reach the CA via nerves. A slow inhibitor, induced by short d, causes reproductive diapause. A fast inhibitor prevents ovarian maturation in starved nondiapausing females. A slow stimulator, induced by feeding under long d, overcomes the fast inhibition of the CA, thereby stimulating vitellogenesis. Food signals are transmitted to the brain via humoral pathways.

Key words: reproduction; photoperiod; feeding; brain; inhibition; stimulation.

INTRODUCTION

The corpus allatum (CA) secretes juvenile hormone (JH) which is essential for vitellogenesis in insects (Wigglesworth, 1970; Engelmann, 1990). CA activity is regulated by external factors, such as photoperiod, feeding, and mating, via the brain. We review the regulation of the CA by photoperiodic and food signals in the adult female of the fire bug, Pyrrhocoris apterus. By comparing the effects of CA regulation in diapause with starvation, we were able to identify slow and fast regulation of the CA by the brain. Although the studies on the regulation of heteropteran reproduction by feeding and mating stimuli indicate that the brain coordinates the function of CA by a combination of inhibitory and stimulatory factors similar to P. apterus (reviewed by Davey [1997]; P. apterus was not included), the question about slow and fast regulatory pathways has not been addressed.

REGULATION OF THE CA IN VIVO

Effect of photoperiod. P. apterus exhibits a facultative reproductive diapause that is regulated by photoperiod, where long-day females develop eggs and short-day females have undeveloped ovaries (Hodek, 1968). Reproductive diapause is characterized by the absence of JH (Denlinger, 1985). A thorough experimental analysis using surgical interventions to the neuroendocrine system in situ and transplantations of parts of the neuroendocrine system between the reproductive and diapausing females revealed that diapause in P. apterus is caused by an active inhibition of the CA from the pars intercerebralis (PI) of the brain. The inhibition can be removed by either denervation of the CA or extirpation of the PI (Hodková, 1976, 1977a, 1977b). Although inhibitory factors are transferred to the CA via nerves in an intact neuroendocrine system, they may occasionally reach the CA via humoral pathways if the denervated CA is close to the brain (Hodková, 1979). The results of the studies on P. apterus are the first record of active inhibition in the regulation of adult diapause. Later, the active inhibition of the CA related to adult diapause was demonstrated in several insect species from different orders, including Heteroptera (Heteroptera: Eurygaster integriceps, Khokhlov, 1977; Plautia stali, Kotaki and Yagi, 1989; Riptortus clavatus, Morita and Numata, 1997; Orthoptera: Locusta migratoria, Darjo, 1976; Horseman et al., 1994; Okuda and Tanaka, 1997; Tetrix undulata, Poras, 1977a, 1977b; Coleoptera: Leptinotarsa decemlineata, Khan et al., 1983; Khan, 1988; Diptera: Protophormia terraenovae, Matsuo et al., 1997). Thus, the active inhibition of the CA by the brain appears to be a general mechanism in the photoperiodic regulation of adult diapause.

Effect of feeding. The CA of nondiapausing starved females of P. apterus, reared under long d, is also inhibited via nervous connections from the brain thereby inhibiting vitellogenesis (Hodková,

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1982). However, the previtellogenic oocytes are about 1.7 times longer in nondiapausing starved females than in diapausing females. The ovaries of allatectomized long-day females have "diapause" appearance irrespective of food conditions (Hodková, 1999). Therefore, the difference in the size of previtellogenic oocytes between nondiapausing starved females and diapausing females may be caused by a higher activity of the CA in the former case. This suggestion is further supported by the finding that the cell volume (Hodková and Karpunina, pers. comm.) and consequently the size of the CA in diapausing females is about half of that in nondiapausing starved females (Hodková, 1999). The complex of brain–subesophageal ganglion–corpora cardiaca–CA (BR–SG–CC–CA) from starved nondiapausing females stimulated vitellogenesis after implantation into allatectomized feeding recipient. Because the complex was not connected to the rest of the nervous system, the inhibition of the CA within the complex was most probably overcome by a humoral factor. In most heteropteran species, the nature of a humoral factor that mediates the effect of feeding has not been identified. However, in Rhodnius prolixus, the feeding stimulus is transmitted to the brain via a peptide hormone originating in the thoracic ganglionic mass (Mulye and Davey, 1995). In contrast to nondiapausing starved females, the complex from diapausing females did not stimulate vitellogenesis after implantation to the same recipient (Hodková, 1992). On the other hand, the isolated CA stimulated vitellogenesis irrespective of photoperiodic or food conditions of donor (Hodková, 1977a). The above data suggest that the inhibition of the CA induced by short d is stronger than that caused by starvation in long d and cannot be overcome by feeding stimulus (Hodková, 1992). An active inhibition of the CA related to starvation was first recorded in Oncopeltus fasciatus (Johansson, 1958).

Effect of mating. Mating has no effect on ovarian maturation in P. apterus. The effect of mating in other Heteroptera females is discussed in detail by Davey (1997).

Regulation of the CA in vitro

Measurement of the CA activity in vitro. The chemical identity of the hormone secreted by the CA in Heteroptera is still unknown (Davey, 1997). Thin-layer chromatography showed that the radiolabel derived from ^{3}H-methionine was incorporated and released by the CA of P. stali as two main products with R_f values of 0.3 and 0.5 that were different from JH I–III or JHB_3. The substance with an R_f value of 0.5 was active in the juvenilizing of P. stali, and its synthesis in vitro was stimulated by farnesoic acid or farnesol (Kotaki, 1993, 1996, 1997). Topical application of CA products, released to incubation medium and extracted by hexane, to allatectomized females stimulated vitellogenin synthesis in P. apterus (Hodková and Kodrík, pers. comm.). The incorporation of radiolabel from ^{3}H-methionine to CA products found at an R_f value of 0.5 was selectively stimulated by brain extracts (Hodková et al., 1996) or farnesol (Hodková et al., pers. comm.) and regulated by intact brains (*see* later). Therefore, the radioactivity found at an R_f value about 0.5 appears to be a valid measure of synthetic activity of the CA.

Effect of intact brain in nondiapausing starved females. Measuring CA activity in vitro confirmed the inhibitory effect of the brain in starved nondiapausing females observed in vivo. The isolated CA showed about two times higher synthetic activity than the BR–SG–CC–CA complex (Hodková and Okuda, pers. comm.).

Effect of intact brain in nondiapausing fed females. In vivo experiments provided no direct evidence for either inhibition or stimulation of the CA by the brain in fed nondiapausing females of P. apterus. The low fecundity recorded in females after extirpation of the PI from the brain could be caused by other effects than suppression of the CA activity; the treatment of females without PI with a JH analog accelerated the maturation of oocytes in the first reproductive cycle, but it did not increase the overall fecundity (Hodková, 1976). Extirpation of the PI or denervation of the CA prevented the growth of the CA cell volume (Hodková, 1977c; Hodková and Karpunina, pers. comm.), but denervation of the CA had no effect on the fecundity (Hodková, 1977b). However, experiments in vitro demonstrated that synthetic activity of the CA increased (about four times, compared to the CA from starved nondiapausing females) only when it was connected to the brain with intact PI during feeding (Hodková and Okuda, pers. comm.). Thus, in fact, both the growth of the CA cell volume and synthetic activity of the CA seem to be stimulated from the PI via nervi allati. This effect of the brain has been termed "slow stimulation" because it requires a relatively long feeding period (several d for maximum effect) and is irreversible during short-term incubation in vitro, i.e., a high activity of the stimulated CA is maintained (Hodková and Okuda, 1996; Hodková, 1999). The inhibition of the CA found in nondiapausing starved females (*see* earlier) was not removed by feeding; the complex of BR–SG–CC–CA from fed females still had synthetic activity that was about four times lower than in the isolated CA. The inhibitory effect of the BR–SG complex in vitro persisted when the nerves between the brain and the CA were cut, although the inhibition was weaker than when the nerves remained intact. The inhibitory effect seems to originate in the PI because it disappears after extirpation of the PI from the incubated BR–SG. The inhibition of the CA in nondiapausing females has been termed "fast inhibition" because it is reversible during short-term incubation in vitro, i.e., it can be quickly removed or restored depending on the absence or presence of the BR–SG or the PI (Hodková and Okuda, 1996; Hodková, 1999). The simultaneous stimulation and inhibition of the CA via nerves in fed nondiapausing females may explain why the small denervated CA (neither inhibited nor stimulated) and the BR–SG–CC–CA complex with the large CA (both stimulated and inhibited) have similar synthetic activities in vitro and permit similar reproductive activities in vivo.

Experimental analysis in R. prolixus led to the conclusion that the inhibition of CA was relieved after blood meal, but a humoral factor probably originating in the brain was essential for the full activity of the CA (Davey, 1997). As the measure of the CA activity was based on egg production, it cannot be excluded that feeding overcomes the inhibition of the CA which still persists in fed females, similar to P. apterus.

Effect of intact brain in diapausing females. In diapausing females, the inhibition of the CA is associated with the suppression of the growth of the CA cell volume in vivo (Hodková and Karpunina, pers. comm.) and is irreversible during short-term incubation in vitro. The synthetic activity of the CA in vitro is negligible irrespective of the presence or absence of the BR–SG in the incubation medium and only slowly increases after denervation of the CA in vivo. Therefore, the inhibition of the CA in diapausing fe-

males has been termed "slow inhibition" (Hodková and Okuda, 1996; Hodková, 1999).

Effect of brain extracts. Extracts of brains from both nondiapausing and diapausing females stimulate synthetic activity of the CA from nondiapausing females. However, there is no direct evidence of a fast reversible stimulation by an intact brain. The CA from diapausing females is refractory to the stimulation by brain extracts (Hodková et al., 1996).

Conclusions

The brain controls the CA activity by two ways (Hodková, 1999; Hodková et al., 2000): (1) the fast inhibition of the synthetic activity under long d prevents vitellogenesis in starved females, and (2) the slow regulation (stimulation and inhibition) of synthetic activity is associated with regulation of the CA cell volume and ultrastructure and mediates the effect of photoperiod. The study on the CA ultrastructure in nondiapausing versus diapausing females of P. apterus revealed considerable differences, e.g., large nuclei separated by abundant cytoplasm versus small packed nuclei, electron dark versus electron lucid mitochondria, abundant endoplasmic reticulum versus lamellar structures indicating degradation of endoplasmic reticulum (Hodková and Cassier, pers. comm.). The slow stimulation by feeding only operates under long d and overcomes the fast inhibition of the CA, thereby stimulating vitellogenesis. The slow inhibition induced by short d keeps the CA refractory to stimulation by feeding, thus preventing vitellogenesis in diapausing females. The fast inhibition of the CA resembles the inhibition of the rate limiting steps by allatostatins (Gäde et al., 1997). On the other hand, the slow regulation of the growth of the CA cell volume might be similar to the regulation of the proliferation and autophagy of cellular organelles and thus regulation of the amount of synthetic machinery in Diploptera punctata (Chiang et al., 1998). Brain factors regulating the growth of the CA in adult insects have not yet been identified. The brain of the last instar larva of a lepidopteran, Manduca sexta, was shown to produce a factor named allatinhibin which has no immediate effect on the CA in vitro, and a prolonged exposure is necessary to inactivate CA in a stable, irreversible manner (Bhaskaran et al., 1990). An allatinhibin has been extracted and partially purified (Unni et al., 1993).

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